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Influence of Altered Local Effective Concentration on Cytoskeletal and Ecm Structure in Human Mesenchymal Stem Cells
Adam S. Zeiger, Felicia C. Loe, Michael Raghunath, Krystyn J. Van Vliet.
Mesenchymal stromal or stem cells (MSCs) are a type of pluripotent precursor cell found in bone marrow, which can be coaxed in vitro to express certain characteristics of differentiated mesenchymal tissue lineages. Many therapeutic applications of MSCs require isolation from a given patient, expansion to useful cell numbers in culture, and reimplantation. However, the in vivo and in vitro culture conditions remain in stark contrast, and confound interpretation of experiments and development of therapeutic uses for MSCs. For example, in vivo, the extracellular microenvironment is highly crowded with proteins; in vitro, the extracellular culture media is decidedly dilute. Here, we use multiple methods to consider whether this differential crowding can directly alter MSC structure and function. We consider a series of macromolecular crowders within the culture media of MSCs, expanded under basal (non-differentiating) conditions. We then quantify changes in population-level proliferation and migration, and in extracellular and intracellular protein structure and cortical cytoskeletal stiffness via immunocytochemistry, optical microscopy, and atomic force microscopy-enabled imaging and nanoindentation of living MSCs. We find significant effects of "crowded" culture conditions on cell architecture, extracellular matrix structure, and attendant cell properties. These results motivate the development of more physiologically relevant environments for both in vitro studies and biodevices, to ultimately answer fundamental questions of physiological and pathological behavior for MSCs.

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Regulating Cell-Substrate Adhesion via Prestress in the Cytoskeleton
Bin Chen, Huajian Gao.
Abstract
We investigate whether and how prestress in cytoskeleton influences cell adhesion with a stochastic-elasticity model of a stress fiber attached on a rigid substrate via molecular bonds. By comparing the variations in adhesion lifetime and observing the sequences of bond breaking with and without prestress in the stress fiber under the same applied force, we demonstrate that the effect of prestress is to shift the interfacial failure mode from crack-like propagation toward uniform bond failure within the contact region, thereby greatly increasing the adhesion lifetime. The present study suggests a feasible mechanism by which cell adhesion could be actively regulated via prestress due to cytoskeletal contractility.

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Tensile Mechanical Characterization of Cell Stiffness Improves Correlation to Metastatic Potential in Models of Osteosarcoma
Guido Bartalena, Yannick Loosli, Tomaso Zambelli, Roman Muff, Jess Snedeker.
A correlative link between decreased cancer cell stiffness and increased aggressiveness (metatstatic potential) has been established in a wide range of cancers, such as leukemia, breast cancer and chondrosarcoma. Nearly all studies establishing this link in adherent cells have utilized cell compression (indentation) or shearing (e.g. magnetic cyto-twisting). In this study we applied a novel tensile assay of cell stiffness to investigate the mechanical behavior of two osteosarcoma models (SAOS-2/LM5 and HUO9/M132), with each system comprising a parental cell line and its highly metastatic derivative form. The goal was to find a common trend linking cell stiffness and metastatic potential, further hypothesizing that a tensile assay could provide additional information compared to traditional compressive measures. Cells were therefore tested in two different mechanical configurations: in compression using atomic force micro-indentation; and in tension using a custom functional imaging platform that applies a controlled bi-axial deformation to the cell substrate while imaging and tracking substrate deformation at high spatial resolution. These measurements were coupled with an inverse finite element model to more precisely estimate cell tensile constitutive behavior.
Both parental lines proved to be significantly ($p<0.01$) stiffer than their metastatic variants in tension: 2.5 ± 1.6 kPa (SAOS-2) vs. 1.4 ± 1.0 kPa (LM5) and 5.3 ± 2.5 kPa (HUO9) vs. 2.5 ± 0.7 kPa (M132). Although a compressive characterization of the SAOS-2/LM5 model confirmed this trend (3.5 ± 1.7 kPa vs. 1.8 ± 0.8 kPa, $p<0.001$), the compressive (cytosolic) stiffness of the HUO9/M132 was unexpectedly higher in the highly metastatic cells (2.0 ± 0.7 kPa vs. 4.9 ± 2.1 kPa, $p<0.001$). These results indicate that the relationship between "cell stiffness" and phenotypic aggressiveness is not straight forward, and that tensional mechanical characterization provides additional, and perhaps more pertinent, information linking cell mechanical behavior to metastatic potential.

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Photobleaching Fluctuations Lead to Apparent Non-Exponential Decay, but can be used to Estimate Number of Fluorophores
Chitra Raju Nayak, Manfred H. Jericho, Andrew Rutenberg.
Intrinsic fluctuations are expected during photobleaching of a fixed number of fluorophores, such as inside a cell. We show that deterministic exponential fits of the stochastic photobleach curves lead to small but significant deviations from the true lifetime and, more significantly, to time-dependent residuals. These time-dependent (non-white) residuals should not be interpreted as evidence for non-exponential decay. We show however that the fluctuations can be directly used to estimate the number of fluorophores per cell, without requiring diffusive fluorophores, and we present an analytic estimate for the quantification error that is confirmed by stochastic simulations. Experimental tests of this fluctuation quantification with calibrated MESF beads leads to the surprising result that the MESF beads exhibit a clear multiexponential behavior that cannot be explained by intrinsic fluctuations. We cannot therefore use these calibrated beads to test our fluctuation-quantification approach.

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Tracking Bacterial Swimming Near a Solid or Air Surface
Liana Nisimova, James Bensson, Guanglai Li, Martin Maxey, Jay X. Tang.
Bacterial motility near a boundary has many implications in surface contamination, biofilm formation, and infection. We study the near surface swimming behavior of microbes using a monotrichous bacterium called Caulobacter crescentus. C. crescentus is a waterborne bacterium that progresses through a two-stage life cycle, alternating between a stationary stalked cell and a motile swarmer cell. The swarmer cell uses its flagellum to propel itself through fluid. We use a non-chemotactic, forward swimming mutant strain lacking pili, SB3860, to focus on near surface motility. Using defocused particle tracking to relate the radius of a dark field image of a swarmer cell to its height away from the microscope's focal plane, we track its motion in three dimensions as a function of time. With this approach, we measure the density distribution from the surface, the speed in relation to height, and the trajectories of bacterial motion. In investigating the behavior of the cells at two different interfaces (water-solid and water-air), we have discovered significant differences in speed, density distribution, and the trajectory shape. At the glass-water interface, a much higher density of cells is found within a micron from the surface, and the average speed reaches a maximum within a few micrometers. At the water-air boundary, however, there is a much wider spread of cell distribution, and the average swimming speed close to surface is significantly lower. Furthermore, most cells at the water-air interface swim in circular trajectories, whereas at the solid-water interface, most trajectories appear straight as the forward swimming cells tend to leave the surface within a fraction of a second. These intriguing properties may be relevant to a variety of microbial functions at the interface, such as accumulation, biofilm formation, and adhesion.

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The Integrins α5β1 and α2β1 Enhance Cell Motility
Claudia Tanja Mierke, Martin Herrmann, Ben Fabry.
Cell migration through connective tissue, or cell invasion, is a fundamental biomechanical process during metastasis formation. Cell invasion usually requires cell adhesion to the extracellular matrix through integrins. In some tumors, increased integrin expression is associated with increased malignancy and metastasis formation. Here, we studied the invasion of cancer cells with different α5β1 and α2β1 integrin expression levels into 3D collagen fiber matrices. Using a cell sorter, we isolated $\alpha5\beta1^{high}$ and $\alpha5\beta1^{low}$ as well as $\alpha2\beta1^{high}$ and $\alpha2\beta1^{low}$ expressing sub cell lines from parental MDA-MB-231 breast cancer cells. $\alpha5\beta1^{high}$ and $\alpha2\beta1^{high}$ cells showed increased cell invasiveness compared to $\alpha5\beta1^{low}$ and $\alpha2\beta1^{low}$ cells, respectively. Similar results were obtained for 786-O kidney and T24 bladder carcinoma cells, and in cells in which the α5 integrin subunit was knocked down using specific siRNA. Knock-down of the collagen receptor integrin subunits α2 did also reduce invasiveness, but to a lesser degree compared to the integrin subunit α5. Fourier transform traction microscopy revealed that the $\alpha5\beta1^{high}$ cells generated 7-fold larger contractile forces compared to $\alpha5\beta1^{low}$ cells. Cell invasiveness was reduced after addition of the myosin light chain kinase inhibitor ML-7 in $\alpha5\beta1^{high}$ cells, but not in $\alpha5\beta1^{low}$ cells, suggesting that α5β1 integrins enhance cell invasion through enhanced transmission and generation of contractile forces.